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The Sunn pest, *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) digestive tract: Histology, ultrastructure and its physiological significance

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ABSTRACT

The Sunn pest, *Eurygaster integriceps* Put. (Hemiptera: Scutelleridae), is a key pest of wheat in the Middle East and some other areas which causes severe qualitative and quantitative damage. The objective of the current work is to describe the morphology of the midgut of *E. integriceps* adult. Microscopic studies revealed that foregut consists of oral cavity, pharynx and oesophagus likely other phytophagous Hemiptera. In the Midgut, four anatomical regions could be identified: the first ventriculus (V1), the second ventriculus (V2), the third ventriculs (V3), and the fourth ventriculus (V4). The microvilli and perimicrovillar membrane (PMM) were found in V1–V3 regions with columnar cells characterized by presence of mitochondria, rough endoplasmic reticulum and basal infoldings in the basal portion. However, V2 and V3 showed less developed basal plasma membrane infoldings. Three cell types: columnar, endocrine and regenerative cells were found in V1–V3. The V4 region showed different histological features from the other three midgut regions by showing a vacuolated epithelium with crypts storing symbiotic bacteria. The hindgut had a short ileum followed by a well-developed rectum with an epithelial cell layer and a thin cuticular intima. The current results suggest V1–V3 midgut regions play a role in enzyme and absorption, whereas V4 seems to have no function in digestion.

1. Introduction

Insects are one of the most successful organisms of the nature, some of them are crop pests causing considerable economic losses to agriculture products. Among the insects, hemipterans are particularly important due to their adaptation capacity and ecological success and due to vectoring serious disease agents to plants (Chapman, 1998; Bandani et al., 2009). Sunn pest, *E. integriceps* (Hemiptera: Scutelleridae) is the most important pest to wheat and barley plantations, its infestation in some areas is near to 100% (Rajabi, 2000).

Morphological studies of the insect midgut are important because this is the organ where digestion and absorption take place (for review see Terra and Ferreira, 2012). In addition, the digestive tract of the insects constitutes the main natural and physico-chemical defense barrier against microorganism invasion that are ingested together with food (Chapman, 1998). Thus, it is a vital system with incredible roles in insect biology and survival. Digestive tract in insects has been co-evolved with their food sources resulting in different structures and tissues development (Terra and Ferreira, 2012). For instance, the protective peritrophic membrane in the midgut of the some insects has been replaced by perimicrovillar membrane (PMM) in Hemiptera (Terra and Ferreira, 2012).

So far, no studies have been done to describe the fine structure of the alimentary canal in *E. integriceps*, although some studies have described the gross morphology of the digestive tract in Hemiptera (Goodchild, 1963) and histological as well as ultrastructural aspects of the midgut cells in *Rhodnius prolixus* (Reduviidae) (Billingsley and Downe, 1986, 1989; Billingsley, 1988, 1990), *Gerris najas* (Gerridae) (Werner et al., 1991), *Dysdercus peruvianus* and *D. fasciatus* (Pyrrhocoridae) (Khan and Ford, 1962; Silva et al., 1995; Damasceno-Sa et al., 2007), *Brontocoris tabidus* (Pentatomidae) (Guedes et al., 2007; Fialho et al., 2009), *Lygus hesperus* (Miridae) (Habibi et al., 2008), *Mezira granulata* (Aradidae) (Nardi et al., 2009) and *Cimex hemipterus* (Cimicidae) (Azevedo et al., 2009).

Some studies regarding *E. integriceps* digestive enzymes have showed that different regions of *E. integriceps* alimentary canal play different roles in enzymes secretion and absorption (Allahyari et al., 2010; Mehrabadi and Bandani, 2011; Mehrabadi et al., 2009).

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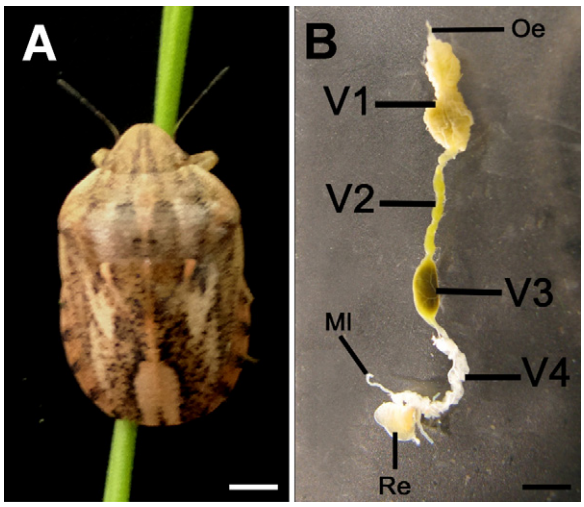

Fig. 1. Adult of *E. integriceps* (A) and its pooled digestive tract (B), showing the first (V1), second (V2), third (V3) and fourth (V4) ventriculus. MI: Malpighian tubules; Re: rectum. Scale bars: 1 mm.

This study investigated the relationship between structure and function of *E. integriceps* alimentary canal and also its physiological significance in digestive tract of *E. integriceps* is proposed (Fig. 1A). It is shown that this bug has typical alimentary canal found in Pentatomorpha with midgut divided into the four distinct regions (V1–V4). The PMM system occurs in the V1–V3 midgut regions but not in V4. In contrast to V4, V1–V3 midgut regions have secretory and absorptive functions. Bacterial symbionts are also present in the lumen of the V4 midgut region and their possible function is discussed.

2. Materials and methods

2.1. Insect

Adults of *E. integriceps* were obtained from the laboratory colonies and maintained on wheat grains at 25 ± 2°C and a photoperiod of 14:10 (L:D) (Allahyari et al., 2010) until dissection. Females were cold immobilized and then dissected in cold 215 mM NaCl solution and the alimentary canals were pulled carefully and rinsed in 0.2 M NaCl for removing the unwanted component. Thus, different parts of alimentary canal were removed for further studies. Ten insects were used for each microscopic analysis.

2.2. Light microscopy

After dissection, the gut samples were transferred to the 4% formaldehyde fixative solution in sodium phosphate buffer 0.1 M, following dehydration in a graded ethanol series (70%, 90%, and 100%) for 20 min each, further 20 min in 1:1 ethanol:xylene, and a final step in xylene for 20 min. Then the samples were embedded in 50% paraffin diluted in xylene at 58 °C for 1 h and mounted in 2 cm^3 moulds. The samples were cut at 5 μm thicker in a Minot microtome, stained with 0.5% toluidine blue and analyzed in a light microscope Olympus BH2.

2.3. Transmission electron microscopy (TEM)

The different parts of alimentary canal were fixed in 2.5% glutaraldehyde, 4% formaldehyde in 0.1 M sodium cacodylate buffer pH 7.2. Following this, the material was rinsed three times in the same buffer, post-fixed in 2% osmium tetroxide and 0.8% potassium ferricyanide for 2 h. Following fixation, samples were dehydrated in a graded acetone series and embedded in epon–araldite resin. Ultra-thin sections (60–70 nm) were cut with an ultramicrotome and mounted on copper grids. The samples were stained in uranyl acetate and lead citrate and analyzed in a Philips CM10 transmission electron microscope.

2.4. Scanning electron microscopy (SEM)

Tissue samples were fixed in 2.5% glutaraldehyde, 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.0, post-fixed in 1% osmium tetroxide, dehydrated in a graded acetone series and critical point dried. Samples were then gold coated and examined using a Zeiss DSM-960A scanning electron microscope.

3. Results

3.1. Gross morphology of E. integriceps alimentary canal

The digestive tract of *E. integriceps* consisted of a narrow and long foregut, an enlarged midgut and a short hindgut. In the transition of mid-hindgut there were some Malpighian tubules (Figs. 1B and 2A). The midgut was divided into four regions (V1–V4) being V4 connected to hindgut (Figs. 1B and 2A). The number of Malpighian tubules varied from four to six. The Malpighian tubules lie in an apparently but not really tangled mass, dorsal to the posterior portion of the midgut, and end blindly. *E. integriceps* midgut lacked gastric caecae. The hindgut had a short ileum followed by a well-developed rectum (Figs. 1B and 2A).

3.2. Histology of E. integriceps alimentary canal

The foregut comprised oral cavity, pharynx and oesophagus. In cross section the oesophagus was oval with the lumen lined by a thick cuticular intima.

The midgut is the large part of *E. integriceps* alimentary canal. The midgut showed a single layered epithelium with columnar cells presenting polymorphic nucleus and a well-developed brush border in the apical surface as well as regenerative cells nidi and isolated endocrine cell scattered among columnar cells (Fig. 2B).

In the midgut brush border and PMMs were present in the V1–V3 regions. The region V1 was the larger *E. integriceps* midgut region, with a sac-like shape providing the enough space for ingested food lined by a columnar epithelium (Fig. 2A and B). The midgut region V2 was tubular with columnar cells presenting brush border and PMMs (Fig. 2C and D). The V3 midgut region was wider and shorter than V2, showing short brush border and few PMMs than V1 and V2 regions (Fig. 2E). The longitudinal and circular muscles were well-developed in the V1 and V2 midgut regions (Fig. 2C). The V4 was the shortest midgut region and was characterized by many sacs or tubular compartments, i.e. crypts, which were arranged in four rows, and fused to each other into a helical assemblage. Crypts lumen was filled by bacterial symbiont (Fig. 2F and G).

The endocrine cells were scattered in the base of the digestive cells and usually occurred between several columnar cells. These endocrine cells were found in the V1–V3 midgut regions (Fig. 2B, D and E). However, their abundance in V1 was more than that in V2 and V3.

The rectum was the mainly hindgut organ, generally filled with fluid and some insoluble feces material and comprised a vacuolated epithelial cell layer and a thin cuticular intima (Fig. 2H).

3.3. Ultrastructure

The oesophagus epithelium had cubic cells onto a thin basal lamina followed by well-developed circular muscles in the